(54) Title: METAL CHELATING PEPTIDE
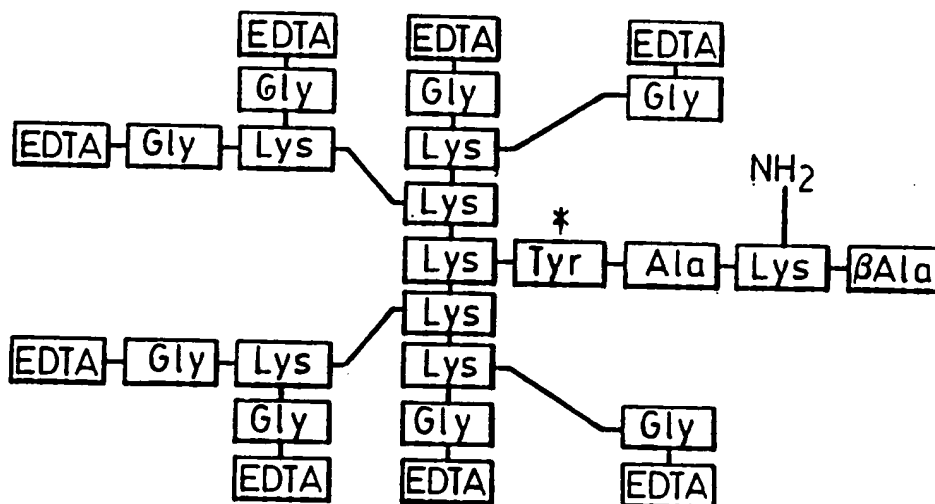
(57) Abstract

The metal chelating peptide (MCP) has a C-terminus which may be structured to provide a variety of means for unidirectional coupling to a targeting agent such as an antibody. The MCP has a branched portion at its other end which contains a plurality of metal chelating sites. The number of metal chelating sites may be quite large (in excess of 16). The MCP of the invention affords the ability to deliver a concentrated radionuclide mass to a target cell by coupling the MCP to a targeting agent.

## METAL CHELATING PEPTIDE

The invention is a metal chelating peptide (MCP)
which can be unidirectionally and selectively coupled to
a targeting molecule such as an antibody.  The peptide of
the invention has a plurality of metal binding sites
attached to a branched portion thereof.

Metal ions including radionuclides may be useful in
diagnosis and treatment of disease.  For example, $\alpha$ or $\beta$
emitting radionuclides are useful for treatment of some
cancers, and diagnostic tumor or thrombus imaging may be
accomplished using paramagnetic metals in conjunction
with magnetic resonance imaging or by using $\gamma$-ray
emitting isotopes for visualization with $\gamma$-ray cameras.
Lanthanide metal fluorescence is also useful in
diagnostic testing, and the ability to create an electron
dense targeting agent for electron microscopy has
applications in basic research.

Delivering metal ions to a desired site, either in
vivo or in vitro, and maintaining a sufficient metal ion
concentration at the desired site for a sufficient time
to accomplish the diagnostic or therapeutic objective,
has been a goal of researchers for many years.  The
invention addresses this need by providing a metal
chelating peptide that may take any of several structures
depending on the intended application.  The peptide has a
reactive group at one end to unidirectionally, i.e.
irreversibly, couple the peptide to the targeting
molecule without affecting the inherent bioselectivity of
the targeting molecule.  The peptide is branched to
provide a plurality of sites for attachment of metal
chelating groups such as ethylenediamine tetraacetic acid
(EDTA), and can be constructed using d-amino acids in
order to resist degradation.  Importantly, the peptides
of the invention are readily synthesized using standard

techniques and may be customized in accordance with the
needs of a particular application.

Typically, the metal chelating peptides of the
invention will be coupled to antibodies, but may also be
coupled to drugs, oligonucleotides, or peptides or other
targeting molecules. In constructing antibody
conjugates, the peptides may be coupled to the $F_C$ domain
of the antibody, thereby not affecting the
immunoreactivity of the antibody with antigen.

Accordingly, the invention provides a metal
chelating peptide which can be coupled with a targeting
molecule. The peptide has a reactive group at one end to
allow unidirectional and selective coupling to the target
molecule, and is branched at the other end to provide a
plurality of metal binding sites. A preferred reactive
group for coupling the peptide is provided by a lysine
residue having a free $\varepsilon$-$NH_2$. Likewise, the branching of
the peptide is preferably provided by a polylysine
construct having free $\alpha$ and $\varepsilon$ $NH_2$ groups to which metal
chelating groups can be attached.

## Brief Description of the Drawings

Figure 1 is a schematic representation of a general
structure for an MCP of the invention.

Figure 2 is a schematic representation of a
particular example of an MCP of the invention.

Figure 3 is a schematic representation of one
possible pathway for coupling an MCP to an antibody.

Figures 4A-F show six possible derivatives of an MCP
for use in various coupling procedures.

While the invention has broad application, it will

be described with reference to a preferred embodiment used for conjugation with antibodies such as monoclonal antibodies. Indeed, the driving force for the creation of metal chelating peptides arose from the desire to produce new generations of radioimmunoconjugates of monoclonal antibodies specific for tumor associated antigens.

With the advent of hybridoma biotechnology in the mid-1970's, there has been a worldwide research effort to develop monoclonal antibodies to tumor associated antigens. In most cases, these antigens either represent altered forms of antigens present on normal cells or are the same antigens but are over expressed on cancer cells. The therapeutic and diagnostic potential of monoclonal antibodies relates to their ability to bind tumor associated antigens. Antibodies themselves may have anti-tumor properties by virtue of immunological mechanisms such as antibody dependent cell mediated cytotoxicity, complement activation, and anti-idiotype activity. However, rapid antigenic modulation and the lack of measurable clinical benefits from the use of antibody alone as a therapeutic agent have caused researchers to turn to an examination of immunoconjugates.

To date, three major families of immunoconjugates have been developed: drug-antibody, toxin-antibody and radionuclide-antibody conjugates. Of these, only the radioimmunoconjugates do not require endocytosis or binding to all tumor cells to deliver their toxic action. Additionally, radioimmunoconjugates are useful diagnostic tools, for example, in locating tumors.

Radioimmunoconjugates have been prepared for both therapeutic and diagnostic purposes; however, the success of such conjugates has been minimal owing to a number of factors which the present invention addresses. One problem arises from the use of monoclonal antibodies

which recognize poorly defined epitopes often located on
the surface of both normal and malignant cells.  Thus,
other targeting agents may prove more suitable for
conjugate formation.  For example, peptide hormones,
growth factors and lymphokines bind to defined cell
surface receptors and may prove to be superior candidates
to antibodies for conjugate construction in many
instances.

Another problem associated with the use of
radioimmunoconjugates is the need for the targeting agent
to retain its binding capacity to a tumor cell marker
after having been modified to carry an effective
therapeutic or diagnostic probe.  Past experience has
shown that antibodies have often been poorly labelled
with radioisotopes, thereby providing radioconjugates
which are too weakly radioactive to be useful, or in
which the metal is not adequately complexed leading to
release of radioactive elements in vivo, or which are
denatured during the conjugation process so that binding
to the target antigen is impaired.

The metal chelating peptides of the invention are
readily synthesized using existing or modified procedures
of classical solid phase peptide synthesis.  In addition
to being rapid and simple, solid phase peptide synthesis
permits the removal of unreacted reagents and by-products
at each step of the synthesis.  Also, each synthetic step
may be repeated until satisfactory yields are obtained.

The peptides of the invention are branched to
provide clusters of metal binding sites.  These peptides
may be customized for various diagnostic and therapeutic
applications in association with a wide range of
targeting agents.

As shown in Fig. 1, the first step in the

construction of a metal chelating peptide of the
invention is to couple at least one amino acid, and
preferably for most purposes several amino acids, to a
solid support for the purpose of providing a molecular
arm X1 which separates the metal chelating portion of the
peptide from the targeting agent coupling portion of the
peptide. This C-terminal end of the peptide X1 has a
reactive moiety permitting the unidirectional coupling of
the peptide to a targeting agent. Thus, the molecular
arm X1 may have a free carboxyl or amide end and may have
a hydrazide, azide, activated ester, thiol, aldehyde,
amino, maleimido, isothiocyanate, haloacetate, photo-
reactive or other reactive group capable of effecting the
desired unidirectional coupling to the targeting agent.

The second part of the peptide of the invention is a
branched structure formed from diamino carboxylic acids
such as lysine, ornithine, or 1,3-diaminopropionic acid.
Referring to Fig. 1, the branched structure is connected
to the C-terminus X1 via the spacer X2 which may be
omitted or may be up to a few amino acids in length
depending on the spacer requirements for separating the
branched portion of the peptide from the targeting agent
coupling site at the C-terminus X1.

In Fig. 1, X3 represents a branch forming amino
acid, preferably a diamino acid such as lysine, ornithine
or 1,3-diaminopropionic acid. The group X4 in Fig. 1
provides the capability for the attachment of a plurality
of chelation sites X5. The preferred branch forming
amino acid is L-lysine; however, the use of D-lysine and
D-amino acids for construction of the peptide will reduce
the rate of degradation of the peptide in vivo, and the
use of shorter diaminocarboxylic acids for branching
produce a more compact structure.

When the desired number of amino groups have been

assembled onto the peptide, a metal chelator X5 is
attached to each amino group.  A preferred metal chelator
is ethylenediamine tetraacetic acid (EDTA), which may be
coupled to the amino end of an amino acid by using the
derivative I, the simple
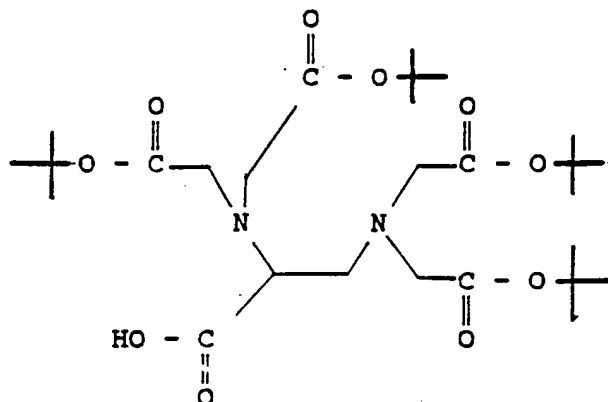


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synthesis of which is reported in Arya, R. and Gariépy,
J. (1991) Bioconjugate Chemistry $\underline{2}$, 323-326.  The
advantage of using an EDTA derivative of the type I is
that the full EDTA structure is available for metal
chelation as a hexadentate ligand.  The skilled person
will appreciate that a similar chelation strategy can be
employed using other chelators such as diethylene-
triamine pentaacetic acid.

    The following is a typical synthetic scheme for
preparation of a peptide of the invention.  The peptide,
termed MCP-8-EDTA-NH$_2$, is shown in Fig. 2.

Preparation of Metal Chelating Peptide, MCP-8-EDTA-NH$_2$

    The peptide shown in Fig. 2 was prepared by solid
phase peptide synthesis on an automated Applied
Biosystems model 430 Peptide Synthesizer using t-Boc
protected amino acids and PAM resin supports.  Unless

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indicated, all couplings were carried out using symmetric
anhydride derivatives of protected amino acids. All
synthesis protocols employed were those established by
the manufacturer (Applied Biosystems, Foster City, CA).
Each coupling step was monitored by the quantitative
determination of free amino groups present on the resin
(quantitative ninhydrin test). Typically, the efficiency
of each coupling step was close to 99%. As illustrated
in Figure 2, the first residue coupled to the PAM resin
was ß-alanine (ß-aminopropionic acid) and the
substitution on the resin support was 0.1 mmole/gram
resin. The initial low substitution value on the resin
insures that crowding of the resin with peptide chains
will not occur as a result of three branching steps (i.e.
maximal substitution of $2^3$ x 0.1 mmole = 0.8 mmole/gram
resin). The ß-alanine serves as an internal standard.
The second residue is a lysine (Nα-Boc, Nε-FMOC-Lysine)
having its ε-amino group protected with a
9-fluoromethoxycarbonyl amino group (FMOC). The base-
sensitive FMOC group provides an option to introduce a
reactive site at this position on the resin prior to
chain cleavage from the resin. A more classical lysine
derivative with an acid labile side chain protecting
group (cleavable with HF; 2 ClZ derivative for example)
has also been used. A protected cysteine residue (for
example Cys (Acm)) could have been introduced at this
position if a thiol group would have been preferred later
for conjugation purposes (for labelling mAb fragments for
example). The third and fourth residues coupled were
alanine and tyrosine respectively. These residues act as
molecular spacers between the peptide branches and the C-
terminus reactive group. They also permit one to monitor
the quality of the synthesis when samples taken at each
step during the construction of the polymer are analyzed
by amino acid analysis. In addition, the aromatic side
chain of tyrosine allows for the determination
spectroscopically the concentration of the peptide stock

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solutions and can be radioiodinated providing an approach
to determine the number of peptides coupled to
antibodies. The first through fourth residues here
correspond to the - X2 - X1 chain of Fig. 1. The fifth
residue was Nα,Nε-Boc-lysine(Boc), an amino acid having
its amino groups at the Cα and Cε positions protected
with acid labile Boc protecting groups. After
deprotecting these sites with TFA, branching is initiated
by coupling two equivalents of Nα,Nε-Boc-lysine(Boc) to
the two available amino positions. After another round
of acid deprotection, the branching step was repeated
with this time four amino sites available for coupling.
Nα,Nε-Boc-lysine(Boc) and glycine(Boc) were successively
coupled again. Upon removal of the Boc groups, eight new
amino sites were exposed. The t-butyl protected EDTA
carboxy derivatives I were then coupled to these amino
sites (2 molar excess over the number of moles of free
amino groups) in the presence of an equivalent molar
amount each of dicyclohexylcarbodiimide and
N-hydroxybenzotriazole in 50% DMF/DCM. The reaction was
performed at room temperature for 2 hours. This coupling
step was repeated and the completion of this reaction as
monitored by quantitative ninhydrin proved to be greater
than 99%. Any remaining unreacted amino groups were
acylated with acetyl anhydride in the presence of
diisopropylethylamine. The t-butyl groups were cleaved
in 50% TFA/DCM for 2 hours and the protecting groups
removed in the presence of trifluoroacetic acid (TFA).
The peptide-resin was then treated with 20%
piperidine/DMF for 20 minutes to deblock the FMOC group
present on the ε-amino group of the C-terminus lysine.
Finally, the MCP-8-EDTA-NH$_2$ (Fig. 2) was cleaved from the
support with 10% anisole/anhydrous hydrogen fluoride at
0°C for 45 minutes. The resin was extracted with several
ether washes to remove the anisole and the cleaved
protecting groups. The branched peptide was then
recovered by extracting the resin with 100% TFA and

rapidly evaporating the acid. The peptide was then
suspended in 50 mM ammonium bicarbonate and lyophilized.

The skilled person will appreciate that the number
of amino groups available for the introduction of
chelator sites can be increased readily to 16 or more,
with the upper limit depending on the ability to resolve
problems of low coupling rates associated with the
crowding of the resin support and the accessibility of
free amino groups. The rapidity of the synthesis
strategy (two days) and its potential for automation
represent major assets in developing the technology.
With an expected molecular weight of 4307 daltons, MCP-8-
EDTA-$NH_2$ is desalted initially on a Sephadex G-10 column
to remove any low molecular weight impurities resulting
from the cleavage step. The recovered polymer has 8
(+/- 2) EDTA groups and the proper amino acid
composition. The chelating properties of MCP-8-EDTA-$NH_2$.
has also been confirmed by the binding of radionuclides
such as $^{99m}Tc$.

Coupling of MCP-8-EDTA to the carbohydrate domain of
monoclonal antibodies

Aldehyde groups can be generated on monoclonal
antibodies by the periodate oxidation of carbohydrate
moieties located in the Fc domain. These sites have been
recently exploited to selectively label antibodies and
glycoproteins with hydrazide derivatives resulting in a
minimal loss of activity.

Introducing a maleimide functionality on MCP-8-EDTA-$NH_2$

A number of strategies can be devised to couple
peptides of the invention to antibodies. This
flexibility is one f the powerful features of the
invention. Fig. 4 illustrates 6 examples of how simple

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modifications of the peptide can lead to alternate
approaches for coupling it to a targeting agent.  In the
selected example outline in Fig. 3, MCP-8-EDTA-NH$_2$ is
first reacted with the bifunctional crosslinking agent,
m-maleimido-benzoyl-N-hydroxysulfosuccinimide ester
(sulfo-MBS; Fig. 3, pathway a).  Typically 3 mg of sulfo-
MBS dissolved in PBS is mixed with 1 mg of the peptide
dissolved in PBS.  The reaction is left to process at
room temperature for 1 hour with occasional stirring.
The polymer-MBS is desalted from unreacted sulfo-MBS by
gel filtration using Sephadex G-25.  Using the reactivity
of the resultant conjugate in a quantitative
DTNB/ß-mercaptoethanol assay, it has been confirmed that
one mole of MBS is coupled to one mole of the peptide
under these conditions.  This compound, abbreviated as
MCP-8-EDTA-NH-MB, is stable for days in aqueous buffers
(PBS) at 4°C.

Construction of MCP-antibody conjugates

     The maleimide derivative of the peptide (MCP-8-EDTA-
NH-MB) selectively and rapidly reacts with any free
thiols present on the surface of targeting agents.  As an
example, thiol groups can be created on an antibody by
partially reducing with DTT one or more of the disulfid
bridges present at the hinge region of IgG molecules.
Alternatively, the Fc domain of antibodies can be
targeted by oxidizing the carbohydrate moieties located
on each heavy chain to create free aldehyde groups (Fig.
3).  Indeed, in the presence of sodium periodate,
aldehyde groups are generated which can then be readily
reacted with a thiol-containing hydrazide compound such
as 2-acetamido-4-mercaptobutyric acid hydrazine (Fig. 3,
pathway b).  The resulting thiol-containing antibody
preparation can then be reacted with MCP-8-EDTA-NH-MB
(50-fold excess in relation to the number of moles of
antibody) overnight at room temperature in an aqueous

buffered solution adjusted to a pH between 4 and 8 (Fig.
3, pathway c). The complex is then d salted on Protein
PakI 125 (Waters; Millipore) HPLC gel filtration column
from the unreacted MCP-8-EDTA-NH-MB.

## Construction of MCP-antibody fragment conjugates

Amino groups on $F(ab')_2$ fragments can be linked
through a bifunctional crosslinker to the MCP-8-EDTA
carrying a cysteine moiety (Fig. 4A as an example), while
the single reactive thiol group of $F(ab')$ fragments will
be coupled to the peptide carrying a lysine group (Fig.
4B as an example). A variety of commercially available
heterobifunctional crosslinkers containing a maleimido
group at one end (reacts with thiol group) and a
succidimidyl ester group at the other (reacts with amino
groups) can be used to link together MCPs and mAb
fragments. In the case of $F(ab')_2$-MCP conjugates, the
antibody fragment will first be reacted with MBS and the
resulting construct purified by gel filtration (Sephadex
G-50). Excess MCPs (50-fold molar excess over amino
groups on fragment) containing a free thiol group is then
added to the desalted fragment-MBS conjugate and the
reaction allowed to proceed for 2 hours at room
temperature (with occasional stirring). In all cases
[$F(ab')$ and $F(ab')_2$ conjugates], the final conjugates can
be purified from the excess polymer by gel filtration
(Sephadex G-50).

## Variations of the basic concept lead to the design of a multitude of MCPs

Fig. 4 illustrates six approaches to link MCPs to
targeting agents. In Fig. 4, only a few variations of
the basic p lymer are shown that would permit an MCP to
be coupled unidirectionally to amino, thiol, or
carbohydrate groups present on a targeting agent. Non-

specific labelling strategies such as the us   of
photoreactive groups (aryl azides or benzoyl benzoate
derivatives, for example, Fig. 4F) are also illustrated.

From the foregoing description and examples, the
skilled person will appreciate that the invention is of
broader scope than the specific peptides illustrated.  A
significant strength of the invention is its versatility
and wide applicability to a variety of metal chelating
applications.

CLAIMS:

1.    A metal chelating peptide, comprising:

      a carboxy-terminal portion having 3-6 amino acid
residues, wherein only the amino acid residue adjacent
the C-terminal residue has a reactive group for
unidirectionally and selectively coupling the peptide to
a targeting molecule; and

      a branched N-terminal portion having 4-16 amino
groups to which are coupled metal chelating polydentate
ligands.

2.    A peptide as claimed in claim 1, wherein the
reactive group is hydrazide, azide, activated ester,
thiol, aldehyde, amino, maleimido, isothiocyanate,
haloacetate or a photoreactive group.

3.    A peptide as claimed in claim 1, wherein the
branched portion is formed by coupling a plurality of
diamino carboxylic acids.

4.    A peptide as claimed in claim 3, wherein the diamino
carboxylic acids are spaced from one another by one to
three monoamino carboxylic acids.

5.    A peptide as claimed in claim 3, wherein the diamino
carboxylic acids are lysine, ornithine or
1,3-diaminopropionic acid.

6.    A peptide as claimed in claim 1, wherein each metal
chelating ligand comprises an ethylenediamine tetraacetic
acid hexadentate ligand after coupling to the peptide.

7.    A peptide as claimed in claim 1, wherein the amino
acid residue adjacent the C-terminal residue is a

diaminocarboxylic acid or cysteine.

8.   A peptide as claimed in claim 1, wherein the amino
acid residue adjacent the C-terminal residue is D or L
lysine.

9.   A peptide as claimed in claim 1, wherein the
C-terminal residue is ß-alanine.

10.  A peptide as claimed in claim 1, wherein the
carboxy-terminal portion is TyrAlaLysßAla.

**AMENDED CLAIMS**
[received by the International Bureau on 7 September 1993 (07.09.93);
original claim 1 amended; remaining claims
unchanged (2 pages)]

1.    A metal chelating peptide, comprising:

a linear carboxy-terminal portion having 3-6 amino
acid residues, wherein said portion has a reactive group
for unidirectionally and selectively coupling the peptide
to a bioselective targeting molecule selected from the
group:  antibodies, antigens, oligonucleoties and drugs;
and

a branched N-terminal portion having 2-16 amino
groups to which are coupled metal chelating polydentate
ligands.

2.    A peptide as claimed in claim 1, wherein the
reactive group is hydrazide, azide, activated ester,
thiol, aldehyde, amino, maleimido, isothiocyanate,
haloacetate or a photoreactive group.

3.    A peptide as claimed in claim 1, wherein the
branched portion is formed by coupling a plurality of
diamino carboxylic acids.

4.    A peptide as claimed in claim 3, wherein the diamino
carboxylic acids are spaced from one another by one to
three monoamino carboxylic acids.

5.    A peptide as claimed in claim 3, wherein the diamino
carboxylic acids are lysine, ornithine or
1,3-diaminopropionic acid.

6.    A peptide as claimed in claim 1, wherein each metal
chelating ligand comprises an ethylenediamine tetraacetic
acid hexadentate ligand after coupling to the peptide.

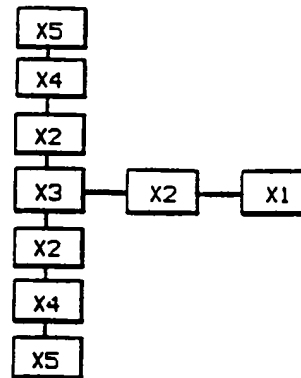7.    A peptide as claimed in claim 1, wherein the amino

acid residue adjacent the C-terminal residue is a
diaminocarboxylic acid or cysteine.

8.　A peptide as claimed in claim 1, wherein the amino
acid residue adjacent the C-terminal residue is D or L
lysine.

9.　A peptide as claimed in claim 1, wherein the
C-terminal residue is ß-alanine.

10.　A peptide as claimed in claim 1, wherein the
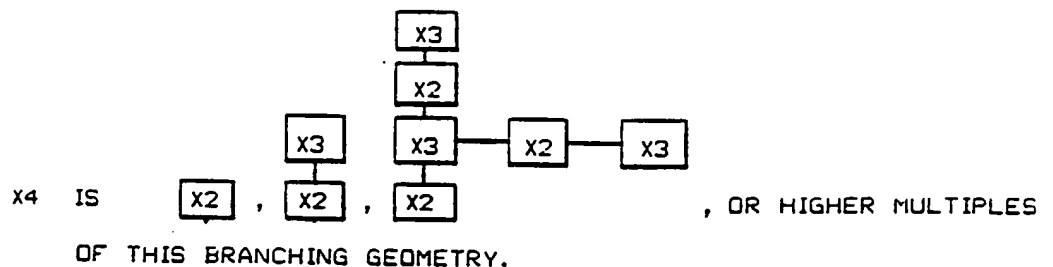carboxy-terminal portion is TyrAlaLysßAla.

WHERE X1   IS AN AMINO ACID, SHORT PEPTIDE OR OTHER MOLECULAR SPACER
           THAT HAS A FREE CARBOXYL OR AMIDE END AND CARRIES EITHER
           A HYDRAZIDE, AZIDE, ACTIVATED ESTER, THIOL, ALDEHYDE,
           AMINO, MALEIMIDO, ISOTHIOCYANATE, HALOACETATE, PHOTOREACTIVE
           GROUP OR OTHER REACTIVE MOIETY PERMITTING THE UNIDIRECTIONAL
           COUPLING TO A TARGETING AGENT.

      X2   IS AN AMINO ACID, SHORT PEPTIDE OR OTHER MOLECULAR SPACER,
           OR X2 MAY REPRESENT NO ATOMS.

      X3   REPRESENTS ANY DIAMINOCARBOXYLIC ACID COMPOUND SUCH AS
           (D OR L) LYSINE, (D OR L) ORNITHINE OR (D OR L)
           DIAMINOPROPIONIC ACID.



X4  IS                                                , OR HIGHER MULTIPLES

    OF THIS BRANCHING GEOMETRY.

X5  IS                              WHERE          REPRESENTS

    METAL CHELATING GROUPS SUCH AS EDTA, DIAMINO DITHIOL
    CONTAINING MOIETIES, OR DTPA.

    n  REPRESENTS THE NUMBER OF FREE AMINO GROUPS GENERATED BY
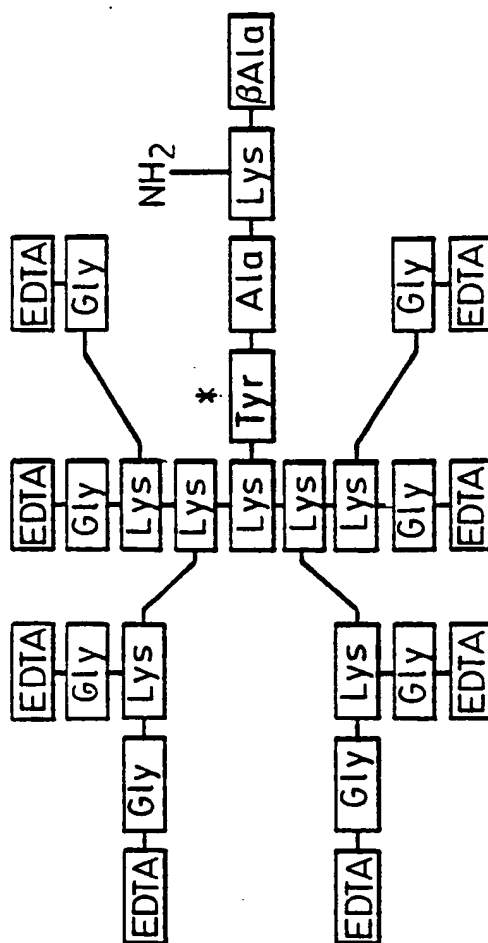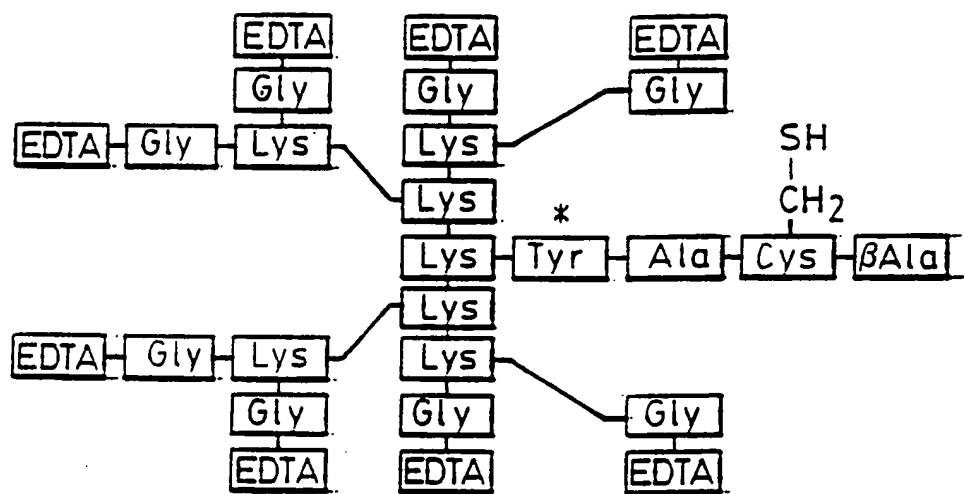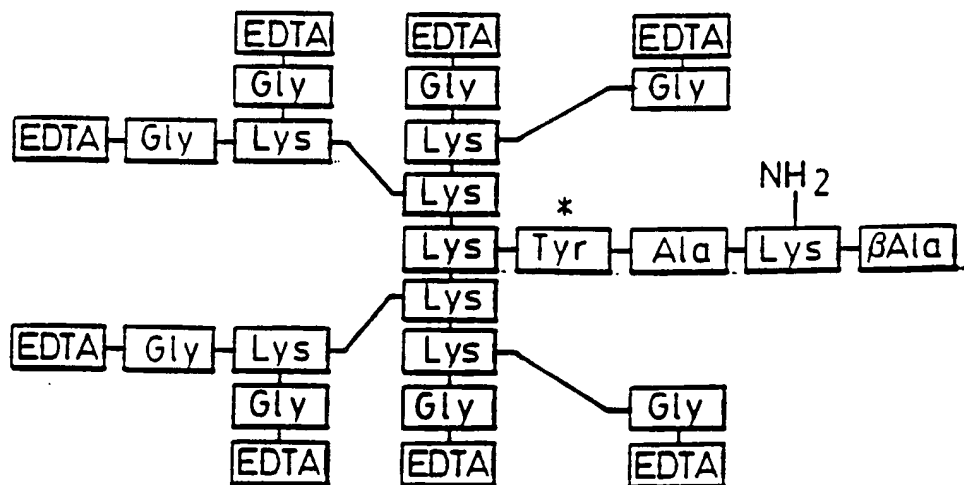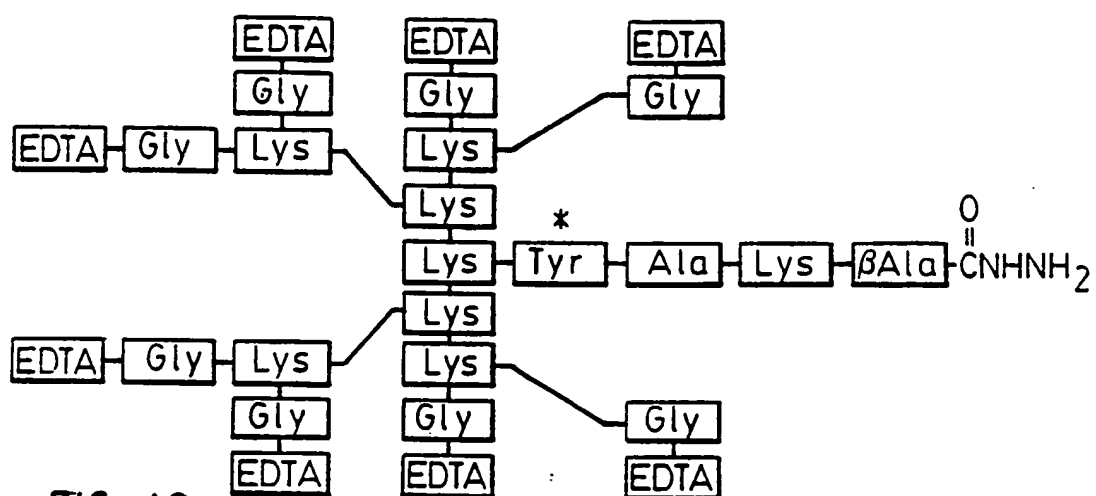    THE PREVIOUS ADDITION OF  X4.

FIG. 1

FIG. 2

FIG. 3

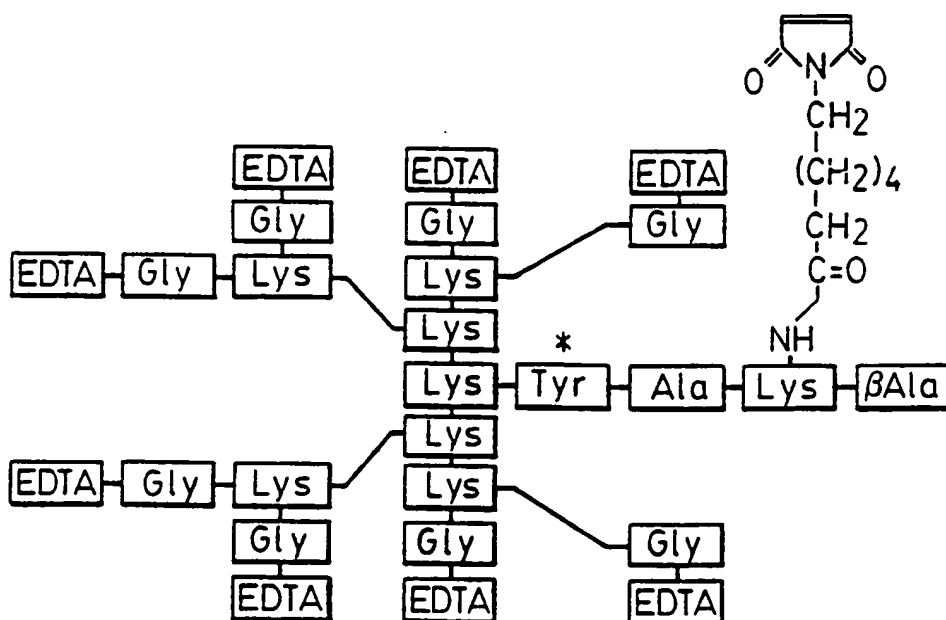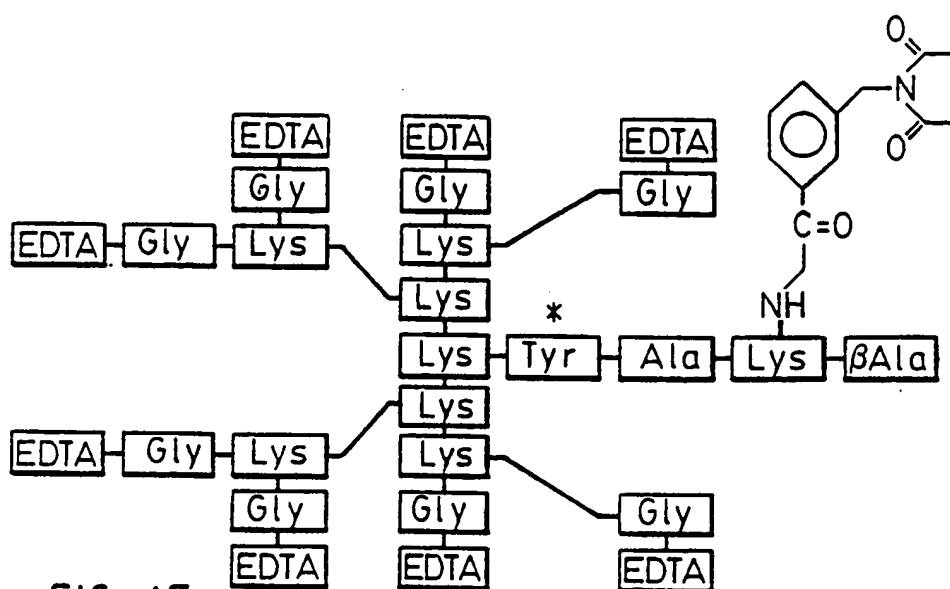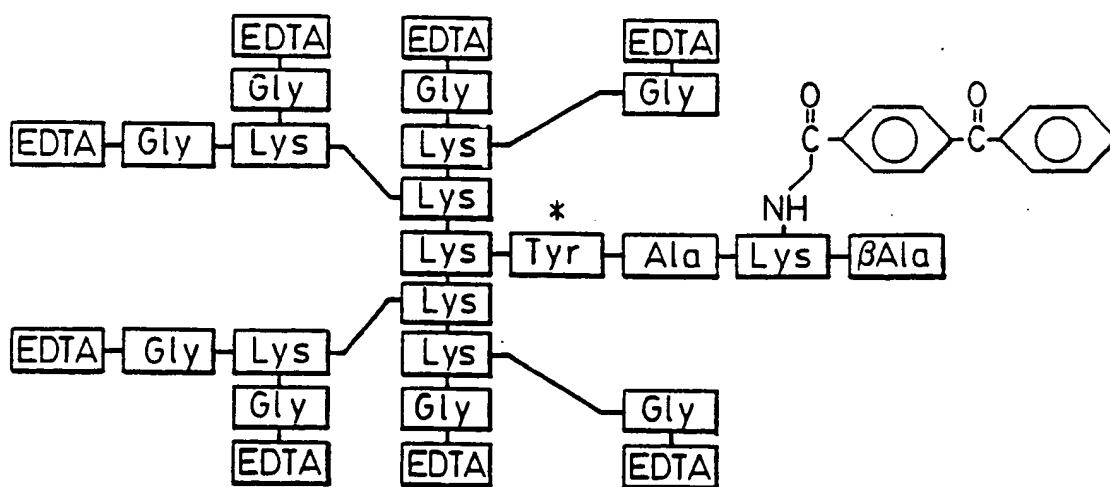FIG. 4A



FIG. 4B

FIG. 4C



FIG. 4D

FIG. 4E



FIG. 4F

**I. CLASSIFICATION F SUBJECT MATTER** (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07K7/04;       G01N33/534;       G01N33/68

**II. FIELDS SEARCHED**

Minimum Documentation Searched[7]

| Classification System | Classification Symbols |
|---|---|
| Int.Cl. 5 | C07K ;      G01N |

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched[8]

**III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]**

| Category[°] | Citation of Document,[11] with indication, where appropriate, of the relevant passages[12] | Relevant to Claim No.[13] |
|---|---|---|
| A | WO,A,9 205 802 (NEORX CORPORATION) 16 April 1992 * pages 1 and 2; figure 1; claim 1 * --- | 1-10 |
| A | EP,A,0 419 387 (IMMUNOTECH PARTNERS) 27 March 1991 --- | |
| A | WO,A,9 101 144 (SANDOZ) 7 February 1991 ----- | |

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

**IV. CERTIFICATION**

| Date of the Actual Completion of the International Search | Date of Mailing of this International Search Report |
|---|---|
| 20 JULY 1993 | 12. 08. 93 |

| International Searching Authority | Signature of Authorized Officer |
|---|---|
| EUROPEAN PATENT OFFICE | HERMANN R. |

## ANNEX TO THE INTERNATIONAL SEARCH REPORT
## ON INTERNATIONAL PATENT APPLICATION NO.

CA 9300207
SA 74506

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.     20/07/93

| Patent document cited in search report | Publication date | Patent family member(s) | | Publication date |
|---|---|---|---|---|
| WO-A-9205802 | 16-04-92 | AU-A- | 8730691 | 28-04-92 |
| | | EP-A- | 0510132 | 28-10-92 |
| EP-A-0419387 | 27-03-91 | FR-A- | 2652004 | 22-03-91 |
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